

Cohen et al. (2022) Aminoglycoside-induced lipotoxicity and its reversal in kidney on chip.

Supplementary Information

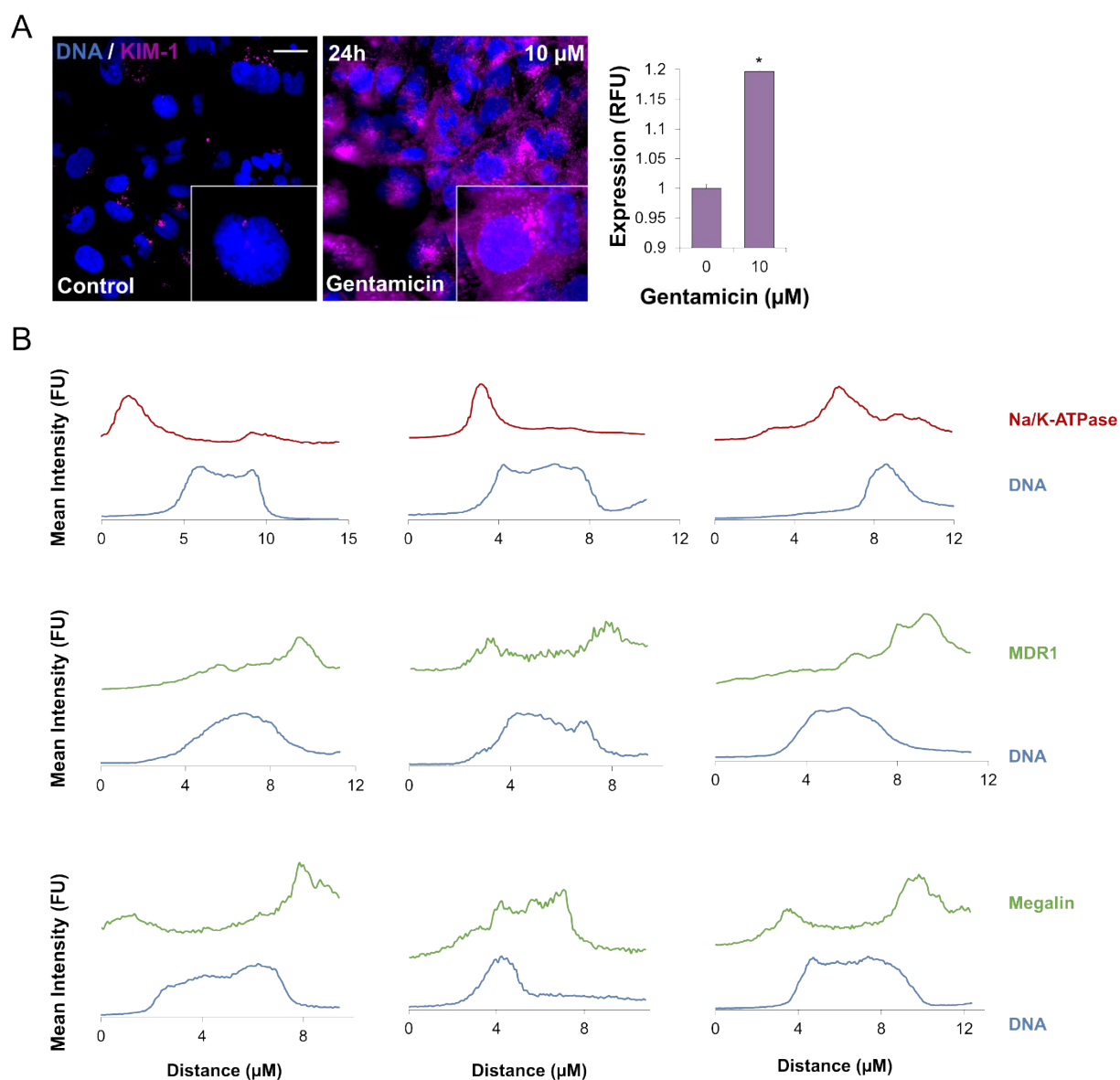
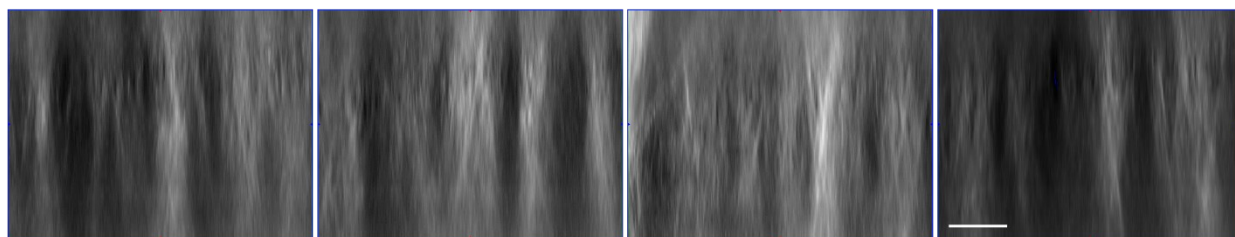
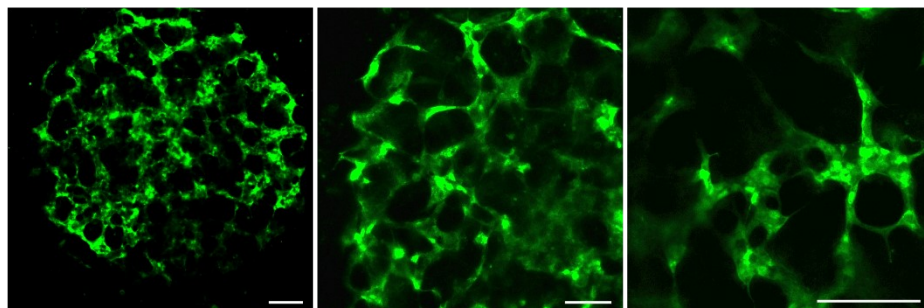


Figure S1. Physiological doses of gentamicin in hPTCs and polarization of hPTC cysts. (A) Immunofluorescence staining of acute kidney injury marker KIM-1 in hPTCs after 24-hour exposure to physiological doses of Gentamicin. **(B)** Traces of transporter distribution in cyst wall showing polarized expression of Na/K-ATPase, Megalin, and MDR1. Bar = 10 μm .

A 3D Confocal reconstruction of LTL-positive verticle tubules



B Confocal cross-section of GFP-labeled vascular structures



C Pressure distribution across organoid surface

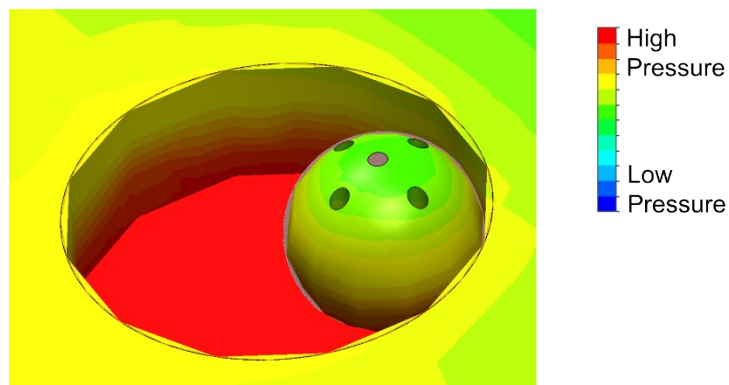
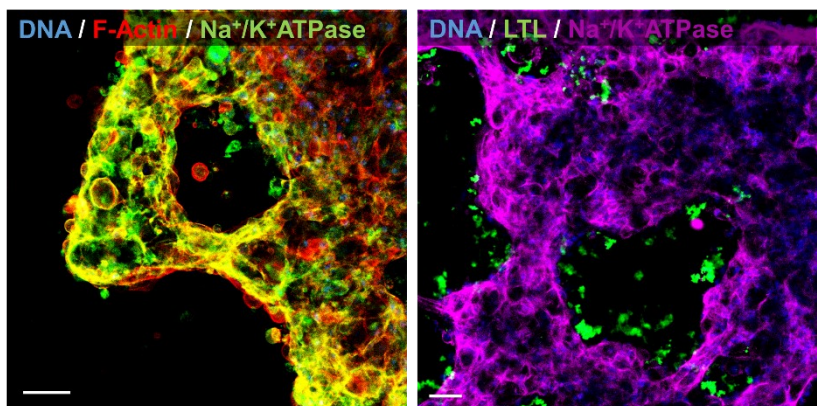


Figure S2. The organization of perfused vascularized 3D human kidney tubuloid. (A) 3D confocal reconstruction showing LTL-positive proximal tubule-like structures assembling tangential to the direction of flow. **(B)** Fluorescence imaging showing the organization of eGFP-labeled endothelial cells in kidney tubuloids. Tubuloid assembly is resolved by day 14, where endothelial networks assemble in direction of flow and hollow tubule-like structures open tangentially. **(C)** Model of pressure distribution in a single well of the kidney on-chip device. A pressure gradient on the opposite side of the organoid is predicted to drive tubular flow. Bar = 100 μm .

A



B

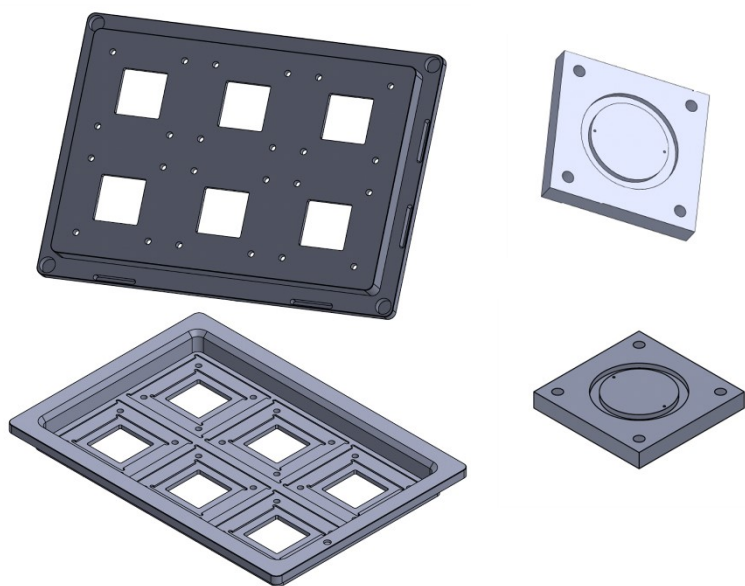


Figure S3. Integration of kidney tubuloids into a Dynamix reader. (A) Confocal microscopy of kidney tubuloids. Lotus tetragonolobus lectin (LTL), and Actin are expressed in tubules ranging in diameter from 20 to 50 μm . Basal surface receptor Na/K-ATPase is pervasive. Bar = 50 μm . **(B)** CAD design of custom inserts for the kidney chip onto a Dynamix platform.

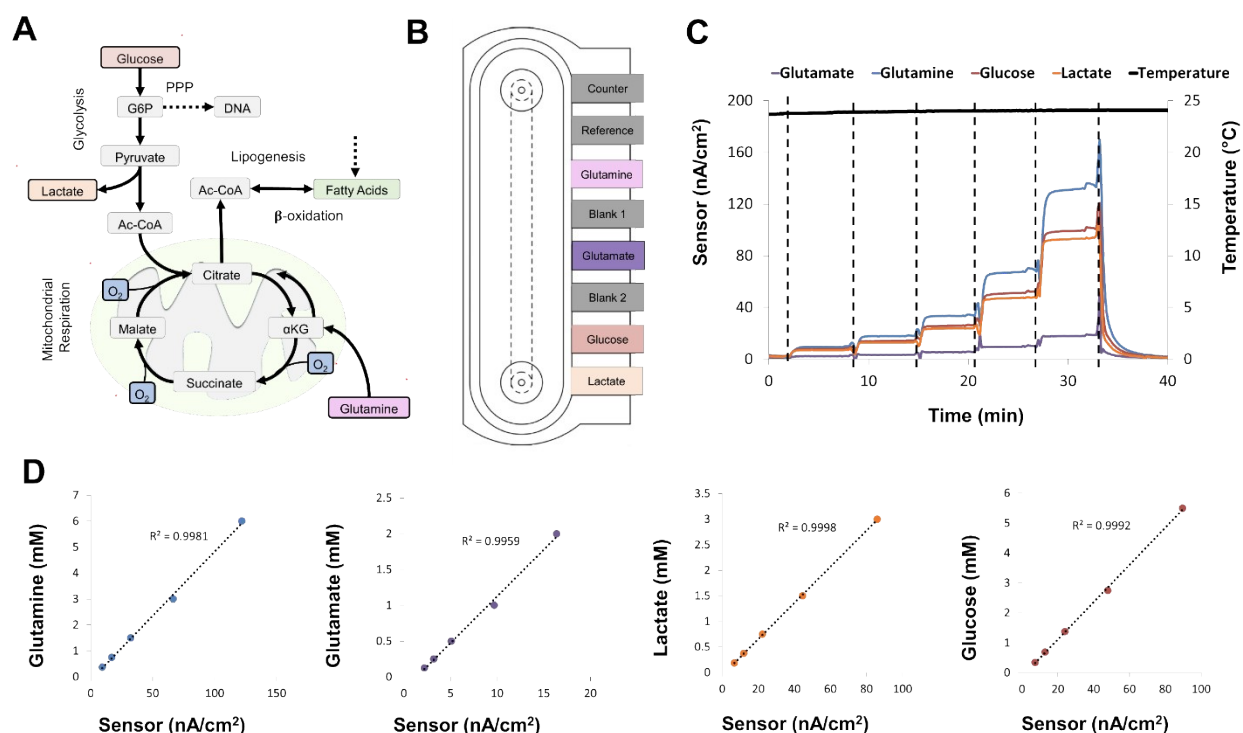


Figure S4. Sensitivity of in-line metabolic sensors. (A) Schematic of central carbon metabolism in human proximal tubule cells. Flux balance analysis permits the calculation of intracellular fluxes (black arrows) using extracellular measurements of oxygen, glucose, lactate, glutamine, and glutamate (red stars). Dotted arrows note experimentally limited fluxes. (B) Low volume microfluidic amperometric, 8-electrode, 4-analytes biosensor array. Anodic oxidation of H₂O₂ on platinum produces a current rapidly ($t_{90} < 25$ sec), while embedded catalase activity prevents cross-contamination. A 450-mV potential between the working and counter electrodes is monitored against a reference electrode to minimize background noise caused by reversible electrolysis events. (C) Raw measurements of glucose, lactate, glutamine, glutamate, and temperature sensors of calibration measurements for different analyte concentrations. Measurements were carried automatically out under a continuous flow of 5 μ L/min. (D) Amperometric calibration curves of glucose, lactate, glutamine, and glutamate concentrations in bioreactor outflow.

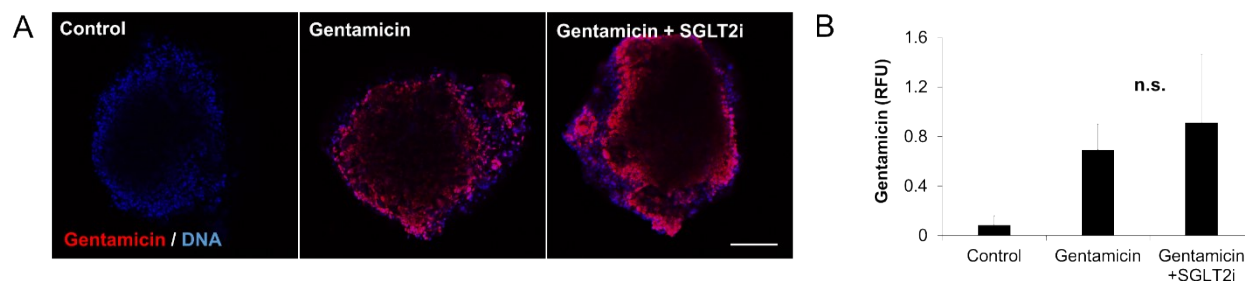


Figure S5. SGLT2 inhibition does not reduce gentamycin uptake (A) Confocal microscopy micrographs and (B) quantification of uptake and transport of rhodamine-gentamicin in tubulids exposed to a physiological concentration of gentamicin for 30 minutes. SGLT2 does not significantly affect gentamicin accumulation. Bar = 100 μ m.

Table S1. List of qRT-PCR primers

Gene	Forward Primer	Reverse Primer
FASN	5'- TTCTACGGCTCCACGCTCTTCC-3'	5'- GAAGAGTCTTCGTCAGCCAGGA-3'
SREBP1c	5'-GCTGTCCACAAAAGCAAATCT-3'	5'-GTCAGTGTGTCCTCCACCTCA-3'
HMGCR	5'-GATGGGAGGCCACAAAGAG-3'	5'-TTCGGTGGCCTCTAGTGAGA-3'
UCP2	5'-GAACGGGACACCTTTAGAGAAG-3'	5'-CCGTGAGACCTTACAAAGCC-3'
CPT2	5'-GCAGATGATGGTTGAGTGCTCC-3'	5'- AGATGCCGCAGAGCAAACAAGTG-3'
COX2	5'-TAGACAGCGTAAACTGCGCCT-3'	5'-TGCCCCACAGCAAACCGTAG-3'
b-Actin	5'-ATCATGTTTGAGACCTTCAAC-3'	5'-CATCTCTTGCTCGAAGTCCA-3'
RPL32	5'- ACAAAGCACATGCTGCCCAGTG-3'	5'- TTCCACGATGGCTTTGCGGTTC-3'

Table S2. List of Antibodies & Probes

Antibodies and probes	Species	Cat. Number	Concentration
Anti-AQP1	Rabbit	Abcam ab168387	1:100
Anti-TIM-1/KIM-1/HAVCAR	Mouse	R&D Systems AF1750	1:100
Anti-megalin/LRP2	Mouse	S. Cruz sc-515772	1:100
Anti-MDR1/ABCB1	Mouse	S. Cruz sc-55510	1:100
Anti-NaK-ATPase	Rabbit	S. Cruz ab76020	1:100
Fluorescein labeled LTL		Vector Laboratories FL-1321	1:50
Rhodamine Phalloidin		Invitrogen R415	1:20
Hoechst 33258		Sigma B2883	1:1000
Anti-Mouse Alexa Fluor 647	Donkey	Abcam ab150107	1:100
Anti-Rabbit Alexa Fluor 488	Donkey	Abcam ab150073	1:100
Anti-Rabbit Alexa Fluor 488	Donkey	Abcam ab150073	1:100